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Corticostriatal and dopaminergic response to beer flavor with both fMRI and [^{11}C]raclopride Positron Emission Tomography

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Abstract

Background—Cue-evoked drug seeking behavior likely depends on interactions between frontal activity and ventral striatal (VST) dopamine transmission. Using [^{11}C]raclopride (RAC) positron emission tomography (PET), we previously demonstrated that beer flavor (absent intoxication) elicited VST dopamine (DA) release in beer drinkers, inferred by RAC displacement. Here, a subset of subjects from this previous RAC-PET study underwent a similar paradigm during functional magnetic resonance imaging (fMRI) to test how orbitofrontal cortex (OFC) and VST BOLD responses to beer flavor are related to VST DA release and motivation to drink.

Methods—Male beer drinkers (n=28, age=24±2, drinks/week=16±10) from our previous PET study participated in a similar fMRI paradigm wherein subjects tasted their most frequently consumed brand of beer and Gatorade® (appetitive control). We tested for correlations between blood oxygenation level dependent (BOLD) activation in fMRI and VST DA responses in PET, and drinking-related variables.

Results—Compared to Gatorade, beer flavor increased wanting and desire to drink, and induced BOLD responses in bilateral OFC and right VST. Wanting and desire to drink correlated with both right VST and medial OFC BOLD activation to beer flavor. Like the BOLD findings, beer flavor (relative to Gatorade) again induced right VST DA release in this fMRI subject subset, but there was no correlation between DA release and the magnitude of BOLD responses in frontal regions of interest.

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Conclusions—Both imaging modalities showed a right lateralized VST response (BOLD and DA release) to a drug-paired conditioned stimulus, whereas fMRI BOLD responses in the VST and medial OFC also reflected wanting and desire to drink. The data suggest the possibility that responses to drug-paired cues may be rightward biased in the VST (at least in right-handed males), and that VST and OFC responses in this gustatory paradigm reflect stimulus wanting.

Keywords

Alcohol; nucleus accumbens; orbitofrontal cortex; ethanol; cue reactivity

Introduction

Drug conditioned stimuli (CS) elicit craving and physiological arousal (Carter and Tiffany, 1999), addiction relapse (Cooney et al., 1997, Grüsser et al., 2004), and promote drug-seeking in animals (Crombag et al., 2008). Given the power of CS to bias behavior toward drug seeking (Berridge, 2007), they remain important in addiction research.

Human fMRI shows that alcohol CS activate striatal and limbic prefrontal areas (Schacht et al., 2013 for meta-analysis), but it remains unclear how limbic frontal areas interact with DA transmission in the ventral striatum (VST). VST DA is widely implicated in addiction-related processes, including abuse potential (Di Chiara and Imperato, 1988), salience attribution (Berridge, 2007), learning (Schultz et al., 1997), and anticipation/craving (Evans et al., 2006, Melendez et al., 2002). The striatum is heavily innervated by glutamatergic prefrontal cortical (PFC) projection neurons (Haber and Knutson, 2010), particularly from limbic areas that process reward and assign value, such as ventromedial prefrontal (vmPFC) and orbitofrontal cortex (OFC). Activation in the vmPFC/medial OFC correlates with imagined reinforcer value at the time of choice (i.e. "goal value", Plassmann et al., 2010), with primary reinforcers represented more laterally and posterior in OFC (for meta-analysis, see Kringelbach et al., 2003). Both the OFC and VST are, in turn, major targets of midbrain dopaminergic projections, with this circuit comprising part of the mesocorticolimbic pathways (Sesack and Grace, 2010). Using positron emission tomography (PET) with the D_2/D_3 radioligand [^{11}C]raclopride (RAC), we previously demonstrated that, in heavy drinkers, the alcohol CS of beer flavor alone (Oberlin et al., 2013), or in combination with alcohol intoxication (Oberlin et al., 2015), causes displacement of RAC in the right VST—usually interpreted as DA release (Endres et al., 1997). Although RAC-PET is useful for tracking striatal DA, it is nevertheless insensitive to neural activity in the PFC, which can be broadly indexed by changes in BOLD (a nonspecific proxy for neural activity; Kwong et al., 1992).

To investigate the relationship between alcohol CS-induced VST DA activity and cortical BOLD changes, we performed an fMRI study in a subset of the parent sample from Oberlin et al., (2013), employing similar flavor cue paradigms in both modalities. Combining data from the current fMRI study with the previous PET study, we hypothesized that beer flavor would: 1) induce activation in right VST, 2) activate medial and bilateral OFC primary reinforcer valuation sites (Plassmann et al., 2010, Kringelbach and Rolls, 2004), 3) produce PFC/OFC BOLD activation that correlated with right VST DA release (from PET), and 4)

increase wanting and desire for beer. To our knowledge, this study is the first to administer preferred alcohol drink stimuli during both fMRI and PET, allowing a determination of the degree to which BOLD responses correspond with VST DA release.

Materials and Methods

Subjects

Twenty-nine healthy right-handed male beer drinkers who previously participated in a RAC-PET study (n=49; Oberlin et al., 2013) underwent a similar paradigm in fMRI 49 ± 38 days later (range 2–160). One subject was excluded for excessive motion in fMRI. Although the RAC-PET data from the parent sample are published, some procedures and data from these (n=28) will be reviewed here for clarity; see Table 1 for subject details. Subjects signed informed consents prior to study procedures, and all procedures were approved by the Indiana University Institutional Review Board. The 90-day Timeline Followback self-report (TLFB: Sobell et al., 1986) from the initial in-person interview for the PET study was used to estimate recent drinking (if > 60 days had elapsed since that interview, the fMRI study day TLFB was used instead). The Alcohol Use Disorders Identification Test (AUDIT: Saunders et al., 1993) assessed alcohol-related problems. The Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994) screened for DSM-IV alcohol use disorder (AUD); two subjects met criteria for probable AUD. Drinking ranged from social to heavy (drinks/week range 2–37). Regular cigarette smoking was exclusionary, although two subjects reported infrequent use (≤ 3 cigarettes or cigars per week).

Procedure

The fMRI flavor paradigm resembled what these subjects had previously experienced during PET (Oberlin et al., 2013 for detail). The PET study, in brief, presented Gatorade® (PepsiCo, Inc., Purchase, NY) and preferred beer in two separate scans (15 flavor trials per scan) using a computer-controlled gustometer. Subjects made subjective ratings (wanting, desire, etc.) after baseline water sprays prior to each PET imaging session, and then again during imaging after 5, 10, and 15 flavor sprays.

The subsequent fMRI paradigm, performed on a later day, delivered Gatorade and preferred beer flavor sprays in six counterbalanced scans (three scans for each flavor; Figure 1). As in the PET study, no alcohol was administered, except for trace amounts in the beer sprays. Individual scans included only one flavor (to mirror the PET procedure) plus intervening water sprays, with 12 flavor and 12 water sprays per scan. While in the MRI scanner, and just prior to imaging, water was delivered to familiarize subjects with the procedure, and to acquire baseline ratings (see below). Subjects' preferred beer was determined during the interview and purchased locally. Preferred beer, Gatorade, and water were chilled with an ice water jacket during administration through the gustometer.

Gustatory stimulus delivery: fMRI

During fMRI, a computer-controlled gustometer and spray nozzle delivered ~0.75 ml of beer, Gatorade, or water onto subjects' tongues, with fluid delivery visually signaled by "Ready 2… 1… Sip" as projected onto a screen. The fluid spray duration was one second,

followed by a 350ms water purge to clear the nozzle head. Flavor and water sprays were delivered with a fixed interstimulus interval of 11 seconds. The general design of the flavor presentation (Figure 1) was chosen to be the best analog of flavor delivery during PET. In fMRI, we acquired multiple but shorter flavor scans with more trial numbers for optimal signal detection within an event related design.

Subjective ratings: fMRI

Subjects responded to computerized rating scales immediately before imaging (baseline), and between each fMRI scan. 'Wanting' was indicated by ratings of the number of beers subjects wanted at the moment (assuming a standard 12 oz. beer), with responses in 0.5 beer increments. 'Desire' to drink alcohol was calculated as the mean of ratings from 4 items from the Alcohol Craving Questionnaire (Singleton et al., 2000) on a 7-point visual analog scale (VAS; 1=strongly disagree, 7=strongly agree). Flavor pleasantness was measured on a VAS (1="Least Pleasant Ever", 7="Most Pleasant Ever"), and flavor intensity was indexed with Green's Labeled Magnitude scale (Green et al., 1996), anchored by "barely detectable" and "strongest imaginable" (labels portrayed on *y*-axis in Figure 2A with proportional from psychophysical scaling, and as seen by subjects in proportion to visual presentation).

Image Acquisition and Processing

RAC-PET acquisition—RAC PET scans were acquired on a Siemens EXACT HR+ (Siemens Healthcare, Erlangen, Germany), with intravenous infusion of 550 ± 39 MBq RAC (mass dose 0.124 ± 0.064 nmol/kg) over 1.5 min, and dynamic acquisition over 45 min (Oberlin et al., 2013).

RAC-PET Processing—In brief, PET frames were registered to each subject's high-resolution anatomical brain volume (see parameters below), and normalized to the canonical Montreal Neurological Institute (MNI) space using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Binding potential (BP_{ND}; Innis et al. 2007) was estimated using the multilinear reference tissue model (MRTM2; Ichise et al., 2003) for all striatal voxels, with the cerebellar time-activity curve as the input function. Voxels with $BP_{ND} \leq 0.75$ were excluded from analyses (Joutsa et al., 2012, Oberlin et al., 2013) by using a conjunct group mask that included only voxels reporting $BP_{ND} > 0.75$ in both conditions in all subjects. Parametric images were smoothed with a 4 mm full width at half maximum (FWHM) Gaussian kernel. The conjunct group mask of all contiguous striatal voxels was eroded by one voxel to minimize edge effects (e.g., spill-out/spill-in). Voxel-wise changes in BP_{ND}, expressed as a percentage of control condition, were calculated as:

$$\Delta BP_{ND} = (BP_{ND[Gatorade]} - BP_{ND[beer]})/BP_{ND[Gatorade]}$$

fMRI acquisition—Functional imaging was performed with a 12-channel head coil array in a Siemens 3T Magnetom Trio-Tim scanner across six echo planar imaging scans (125 BOLD volumes, 2250/29ms repetition/echo time, 78° flip angle, 2.5×2.5×3.0 mm^3 voxels, 220×220 mm field-of-view, GRAPPA acceleration factor 2). Head motion was minimized with deformable foam pads on both sides of the participants' head, and by employing a real-

time prospective acquisition correction (Thesen et al., 2000). T1-weighted 3D Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE; 160 sagittal slices, 1.0×1.0×1.2 mm^3 voxels) images were acquired for transforming the BOLD volumes into MNI stereotactic space.

fMRI Processing—SPM8 pre-processing included slice-timing acquisition correction, rigid-body realignment, segmentation of and co-registration to subjects' own high-resolution anatomical brain volume, transformation to MNI space (2 mm/side voxels), and 6 mm FWHM isotropic Gaussian kernel smoothing.

Residual head motion during BOLD scans was evaluated using the ArtRepair toolbox version 5b http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html, (Mazaika et al., 2009). BOLD volumes with large (> 1.4%) volume-to-volume global signal intensity changes were classified as outliers and individual flavor scans with more than 40% outlier volumes were excluded from subsequent analyses. Based on this criterion, one subject's entire fMRI dataset was discarded yielding the final n=28 sample. Twenty-five subjects provided 3 beer and 3 Gatorade scans, with the remaining three subjects each contributing 2 beer and 2 Gatorade scans. The percentage of outlier volumes in the final sample did not differ between beer and Gatorade scans, 9.2 ± 9.7% and 9.2 ± 8.5%, respectively; $p > 0.9$ by t-test.

Statistics: Ratings

Mean ratings in PET and fMRI were tested with repeated measures ANOVA (Modality × Flavor). Only the ratings from the fMRI experiment are reported here (unless a significant main effect of Modality was detected), as PET ratings were previously described (subsample of n=49; Oberlin et al., 2013). Detection of significant effects in ratings during fMRI were followed by paired t-tests between flavors. All in-text means are plus/minus the standard deviation unless otherwise noted.

Statistics: Imaging

Regions of interest (ROIs)—In the parent sample, the right VST (but not the left) showed a DA response to beer flavor, so this region was used to assess BOLD activation and any correlations between BOLD and other variables of interest. Three *a priori* ROIs were defined in all: 1) the same anatomical right VST ROI (A-P center at y=12) used in Oberlin et al., (2013) that showed a CS-elicited DA response to beer flavor; 2) left and right OFC (two 8 mm radius spheres, excluding white matter, centered on [±24, 30, −16]; Kringelbach and Rolls, 2004, Kareken et al., 2013), identified by meta-analysis as sensitive to primary reinforcers; 3) medial OFC/ventromedial PFC (8 mm radius sphere centered on [0, 32, −20]), where responses are thought to reflect "goal value" (Plassmann et al., 2010, Plassmann et al., 2007)—the imagined value of a reinforcer at the time of choice. Peaks in these *a priori* regions were considered significant at $p_{FWE}<0.05$, as corrected by region volume. Individual BOLD flavor effects, that is ([beer > water] and [Gatorade > water]), are presented in Supporting Information for completeness, but not included in the primary analyses (which instead focuses on differential responses between beer and Gatorade).

PET: DA response to alcohol-paired cues—The RAC-PET data were a subset (n=28) of a larger group (n=49), which showed a right VST DA response to beer flavor (Oberlin et al., 2013). The analysis of the current n=28 subset used the same anatomically-defined *a priori* right VST region to identify voxels in which ΔBP_{ND} was significantly greater than zero (one-sample t-test).

fMRI: BOLD responses to alcohol-paired cues—Within-subject fixed effects of BOLD response to fluid delivery trials were estimated using SPM's canonical hemodynamic response function, with an autoregressive AR(1) model accounting for serial correlations. The six movement parameters from realignment were included as regressors, and a high-pass filter (1/128 Hz) removed low-frequency noise. As each scan captured one flavor plus water, use of the [flavor > water] contrasts minimized between-scan baseline drifts of the BOLD signal. To maximize flavor-water differentiation, water sprays immediately following a flavor spray (3 water sprays per scan) were separately modeled due to concerns about residual flavor effects (see Kareken et al., 2013). The [beer > water] and [Gatorade > water] contrast differences were tested against zero with SPM8's one sample t-test. This allowed us to compare beer and the appetitive control [beer > Gatorade] responses, by contrasting each against the within-scan water baseline. Drinking behavior (at the time of interview), i.e. Drinks/week, drinks/drinking day, heavy drinking days/week, AUDIT, and self-reported wanting and desire were tested for correlations with the [beer > Gatorade] response. Drinking behavior assessed on the fMRI study day was also tested (note that four subjects whose fMRI study day was within 10 days of the PET study were not administered new TLFBs.) Craving measures were the differences between the ratings during beer flavor and Gatorade flavor.

PET-fMRI Correlation—For each subject, mean ΔBP_{ND} values were extracted from the responding region within right VST in PET for subsequent voxel-wise correlation with BOLD fMRI in SPM8 constrained to our identified regions of interest. To assess possible effects from other factors, we added other covariates separately: 1) PET-fMRI delay time in days, 2) drinks/week, 3) drinks/drinking day, and 4) heavy drinking days/week. To explore all possible correlations of imaging measures from both modalities within the right VST, we also extracted $BP_{ND[Flavor]}$ (PET) and [flavor > water] contrast values (fMRI) to assess correlations between mean ROI values from each modality (e.g. between $BP_{ND[beer]}$ and [beer > water]); results reported in Table 3.

Results

Stimuli

The total fluid volumes delivered were 26.3 ± 2.3 (beer), 29.7 ± 3.3 (Gatorade), and 97.2 ± 9.0 mL (water). Slightly less beer was delivered than Gatorade (mean difference: 1.1 ml/scan; $t(27) = 7.2$, $p < 0.001$), which we attributed to residual carbonation in the beer.

Subjective Ratings

Stimulus qualities—Subjects rated beer and Gatorade flavors as more intense than water $ts(27) > 7.0$, $ps < 0.001$, but their perceived intensities did not differ from each other ($p>0.8$),

Figure 2A. Beer was not more pleasant than water (p=0.6), but Gatorade was perceived as more pleasant than water or beer (ts(27) > 2.2, ps < 0.037), Figure 2B.

Wanting for beers and desire to drink—Beer flavor and Gatorade both increased number of beers wanted (ts(27) > 2.4, ps < 0.022), but beer flavor had a greater effect than Gatorade (t(27) = 2.9, p = 0.007). Desire to drink showed a similar pattern (ps < 0.012), Figure 2C.

Flavorants were more pleasant in PET (beer= 5.0 ± 1.1; Gatorade= 5.4 ± 0.9) than fMRI (beer= 4.5 ± 1.2; Gatorade= 5.0 ± 1.0; ts(27) > 2.0, ps < 0.05). Other ratings did not differ by modality.

fMRI: Whole brain flavor effects

flavor > water—Both beer and Gatorade flavors, compared to water, activated primary gustatory cortex (anterior insula/frontal operculum), amygdala, and caudate; although beer activation was bilateral while Gatorade activation was weaker and left-dominant. In contrast, only beer flavor activated OFC. These results are illustrated in *Supporting Information* Figure S1 and detailed in Tables S1 and S2.

fMRI: Alcohol CS effects

beer > Gatorade—Compared to Gatorade, beer flavor showed greater activation in the right VST (peak voxel at [6, 6, −4], Z = 3.19, p_{FWE} = 0.029) and bilateral OFC, with the peak in the right OFC ([22, 36, −14]) achieving corrected significance (Z = 3.39, p_{FWE} = 0.038); while the left OFC peak reached only an uncorrected p_{uncorr} = 0.001 height, figures 3A and B. No effects were detected in the medial OFC or other ROIs for the opposite contrast of [Gatorade > beer]. Whole-brain effects of appetitive flavor contrasts are shown in Table 2.

fMRI: Correlated factors

The [beer > Gatorade] BOLD contrast correlated positively with “number of beers wanted” in right VST ([6, 16, −4], Z = 3.54, p_{FWE} = 0.011) and medial OFC ([−6, 30, −18], Z = 4.78, p_{FWE} < 0.001); Figure 4 A–B. Similarly, desire to drink correlated positively with the BOLD contrast in right VST ([6, 16, −4], Z = 3.10, p_{FWE} = 0.037) and medial OFC ([−2, 26, −16], Z = 4.33, p_{FWE} = 0.001; not illustrated). Neither negative correlations, nor correlations with recent drinking/problems, were present for either interview-day or fMRI study day recent drinking (TLFB).

PET: DA release in response to beer flavor

Consistent with the parent sample results (n=49; Oberlin et al., 2013), beer flavor in this subsample significantly increased DA relative to Gatorade (ΔBP_{ND} > 0; n=28; [8, 14, −6], Z=3.12, p_{FWE} = 0.021), Figure 5. ΔBP_{ND} was 5.5 ± 8.8% in the cluster formed by voxels exceeding p<0.01 within the right VST, and 3.3 ± 7.7% for the entire anatomical right VST region. For comparison, ΔBP_{ND} was 0.2 ± 8.0% in the left anatomic VST.

fMRI: DA correlations

The [beer > Gatorade] BOLD contrast was neither positively nor negatively correlated with right VST ΔBP_{ND} in any of the fMRI search regions. Including the time between the PET and fMRI scan days and the drinking variables did not change this outcome. The non-significant relationships between the mean right VST values during PET (binding potential) and fMRI (BOLD contrast) are presented in Table 3 for completeness.

Discussion

This multi-modal imaging study sheds new light on the relationships between drug cue-induced human limbic frontal activity and VST dopamine changes. As hypothesized, alcohol flavor cues evoked right VST and OFC BOLD responses in fMRI while enhancing wanting and desire for beer. Medial OFC, a locus of reinforcer valuation, positively correlated with wanting and desire for beer. BOLD responses to beer flavor did not, as hypothesized, correlate with right VST DA release to beer flavor in this subset of subjects from a larger RAC-PET sample.

A large body of literature implicates the VST in aspects of CS-signaled reward anticipation (Berridge, 2007). The VST (right in particular) shows activation to alcohol CS and reduced alcohol cue-elicited activation after treatment across a range of behavioral and pharmacotherapies (Schacht et al., 2013 for meta-analysis) suggesting its importance in clinical outcomes. The VST is positioned at the nexus of descending cortical information regarding reward motivational states and action planning that either facilitates or inhibits reward seeking (Sesack and Grace, 2010). For example, retro- and anterograde tract tracing in monkeys (Haber et al., 2006) showed that the VST receives substantial input from the OFC, which codes primary reinforcers and reward value (Kringelbach et al., 2003, Plassmann et al., 2010), and mediates reward learning (Clark et al., 2004). Furthermore, human VST and OFC are functionally coupled at rest (Di Martino et al., 2008). Germane to addiction, the higher order learning that leads to CS enhancement of operant reward seeking (e.g. Pavlovian-to-Instrumental Transfer) relies on the OFC (Ostlund and Balleine, 2007).

We demonstrated that an alcohol flavor CS enhanced motivation to drink alcohol more than an appetitive flavor control. Both imaging modalities showed that the alcohol CS altered right-sided VST activity by increasing the BOLD response (fMRI) and inducing DA release (PET). These findings are consistent with the incentive sensitization hypothesis (Berridge, 2007), which posits that VST activity reflects drug wanting. However, it was also case that the number of beers wanted and the desire for “a drink” correlated with activity in the ventromedial OFC, a region that both projects to the VST and codes for subjective valuation (Plassmann et al., 2010, Hare et al., 2009). Our findings thus cohere with a neuro-behavioral literature that implicates these frontal limbic and striatal dopaminergic systems in the motivational processes that govern addiction behaviors.

Few alcohol cue-reactivity studies have employed actual preferred alcohol drinks during imaging, which is arguably the most proximal and best learned cue for testing conditioned responses to an orally-consumed, flavored liquid drug. Two notable fMRI studies that did administer preferred alcohol drinks during scanning demonstrated both striatal and

vmPFC/OFC activation to alcohol-flavor CS (Claus et al., 2011, Filbey et al., 2008) compared to control. The latter study showed that craving correlated with activation to alcohol cues in the right OFC. Although our results generally align with these findings, the main effect of [Alcohol cue > control] in the Claus et al. (2011) study was dorsal, rather than ventral striatal, and the Filbey et al. (2008) correlation results were more right-lateralized in OFC than ours. Of note, these prior studies used lychee (litchi) juice as an appetitive control; this flavor may be a novel taste for many Westerners and could conceivably affect the localization of the neural responses.

The current fMRI results of right-dominant BOLD response in the VST mirror both the current DA results, as well as our prior findings. Using RAC-PET in a separate sample of heavy beer drinkers (n=26), we demonstrated DA release in right VST (but not left) to beer flavor cues during alcohol intoxication (Oberlin et al., 2015), suggesting a special role for the right VST in responding to drug-paired cues. In addition to meta-analytic evidence of a right-lateralized VST response to alcohol cues (Schacht et al., 2013), other support comes from two prior fMRI studies indicating that right VST responses to alcohol cues are attenuated by treatment with naltrexone and ondansetron (Myrick et al., 2008) and aripiprazole (Myrick et al., 2010). However, this paradigm (a sip of alcohol, then visual alcohol images during scanning) did not always elicit striatal responses (Myrick et al., 2004). Gender may modulate lateralization of VST DA responses, as one study using unanticipated monetary reinforcers showed right VST DA response in men, but bilateral effects in women (Martin-Soelch et al., 2011). The all-male composition of the current study and Oberlin et al. (2015), along with the 73% (combined) male composition of Myrick et al. (2008, 2010) leaves open the question of potential gender-by-hemisphere interactions of VST responses to drug cues.

We did not detect the hypothesized correlation between ΔBP_{ND} and BOLD responses to beer flavor, even when the delay between the two types of scans and the subjects' drinking behavior were taken into account. Reports of significant relationships between dopaminergic measures from PET and BOLD brain activity vary greatly across the literature. A similar multimodal study of alcohol cues in 11 alcoholics and 13 healthy men failed to detect correlations between baseline BP and BOLD response to alcohol cues in the VST (Heinz et al., 2004); however, it did detect correlations between baseline VST BP and BOLD responses in rostral anterior cingulate and mPFC in the alcoholic group. The Heinz et al. study differed from the present study's findings in several important ways: 1) they found dopaminergic-BOLD correlations in alcoholic subjects only, and not controls, 2) they did not conduct a cue challenge study in the PET paradigm, and 3) the correlations were only with baseline VST DA D_2 availability. There are two multimodal studies with the monetary incentive delay task in which ΔBP_{ND} and BOLD responses correlated during feedback indicating winning (Weiland et al., 2016) or anticipation of reward (Schott et al., 2008). The former study found correlations between left nucleus accumbens ΔBP_{ND} and BOLD responses in mPFC, superior frontal cortex, and several other cortical areas, but not the nucleus accumbens. The latter study found that for reward anticipation, ΔBP_{ND} in the left nucleus accumbens correlated with left nucleus accumbens BOLD. This was established by using the peak effect coordinates in PET [−6, 10, −6] to locate the nearest local maxima for placing individualized 6mm radius spheres from which the mean BOLD values were then

extracted. Therefore, the data used for the multimodal comparisons did not sample precisely the same space within the VST of each subject, and potentially included non-VST contributions.

The lack of the hypothesized ΔBP_{ND}–BOLD correlation in our data may not be unexpected, as brain areas affected by the VST may not respond in a 1:1 manner to DA release (even though group effects from both DA and BOLD were each present in the right VST). Indeed, BOLD signal changes (reflecting a sum of neural events; Kwong et al., 1992) and ΔBP_{ND} (an indirect measure of endogenous neurotransmitter displacement; Endres et al., 1997) do not measure precisely the same type of neural event, and may be only loosely correlated. Our power to detect ΔBP_{ND}–BOLD correlations may also be limited by the modest magnitude of inferred DA release. Specifically, the subjects who agreed to return for fMRI showed a more limited dynamic range of ΔBP_{ND} than did the parent sample. Urban et al., (2012) were similarly unable to detect correlations between RAC-PET and fMRI and also attributed the absence of such a relationship to the small effect sizes in ΔBP_{ND}.

Some considerations temper our interpretations. Although the PET and fMRI paradigms were designed to be as similar as the corresponding modalities would permit, they were not perfect analogs. Pleasantness ratings were lower in fMRI than PET, an effect we attribute to the larger number of flavor sprays in fMRI (72 vs. 30 in PET). Although a sample size of 28 is reasonable for fMRI, previous cue reactivity studies obtained greater power with larger samples (Claus et al., 2011), albeit without data that speak directly to DA release. Our fMRI results are in general agreement with similar prior studies, and also add novel information about relationships between limbic prefrontal reward/valuation regions and cue-induced VST DA release. Finally, the study was limited to men (due to the difficulty of recruiting nonsmoking female heavy beer drinkers).

In conclusion, we believe this to be the first multi-modal demonstration in humans of alcohol cue related BOLD and DA responses. The results support the idea that (right) lateralized VST may be of special import to addiction research. Although such *in vivo* approaches remain indirect measures of neural activity, we hope that studies like these will be performed in larger cohorts and extended to further clarify how the neural circuits subserving drug-related cue associations contribute to the development and maintenance of alcoholism in both sexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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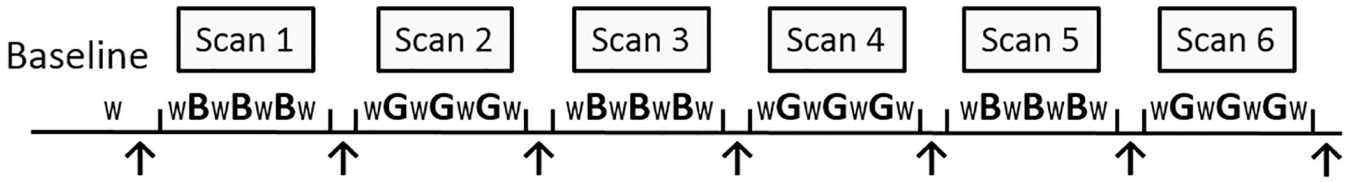

Figure 1. fMRI: Paradigm

Following a water baseline, six scans alternated beer or Gatorade flavor administration, with water interspersed within-scan. Subjective ratings followed the water baseline and each scan, indicated by vertical arrows (↑). Scan length=4:48, w=3 water sprays, B=4 preferred beer sprays, G=4 Gatorade sprays. Spray vol. ~0.75 ml each; flavor order counterbalanced between subjects (beer first shown here).

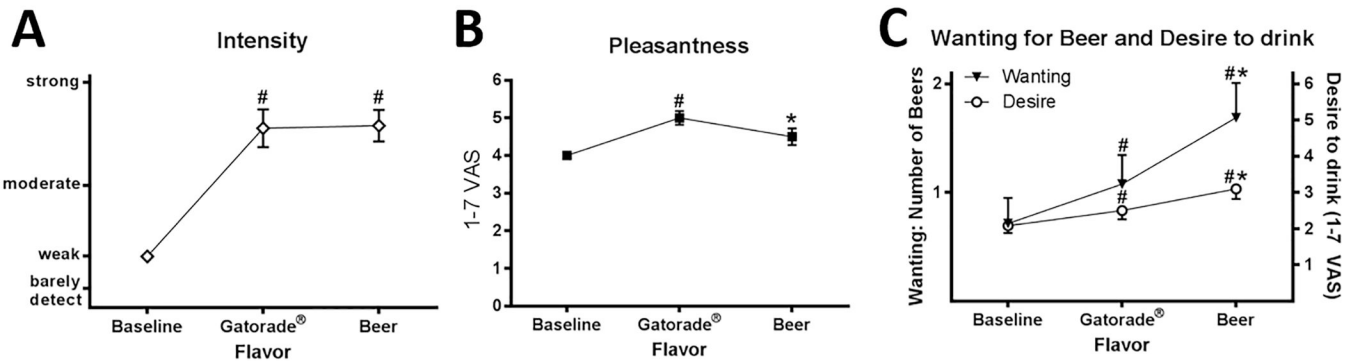

Figure 2. fMRI: Subjective ratings

Subjects (n=28) rated perceptions of flavor stimuli and wanting/desire for beer. (A) Beer and Gatorade were perceived as equally intense; note that the y-axis mirrors the rating scale. (B) Beer flavor was less pleasant than Gatorade, but similar to water. (C) Beer flavor increased wanting for beer and desire to drink. Baseline (water) was rated before scanning; beer and Gatorade ratings shown here are means of three ratings collapsed across scans of the same tastant. VAS = visual analog scale, #p<0.05 compared to water, *p<0.05 compared to Gatorade.

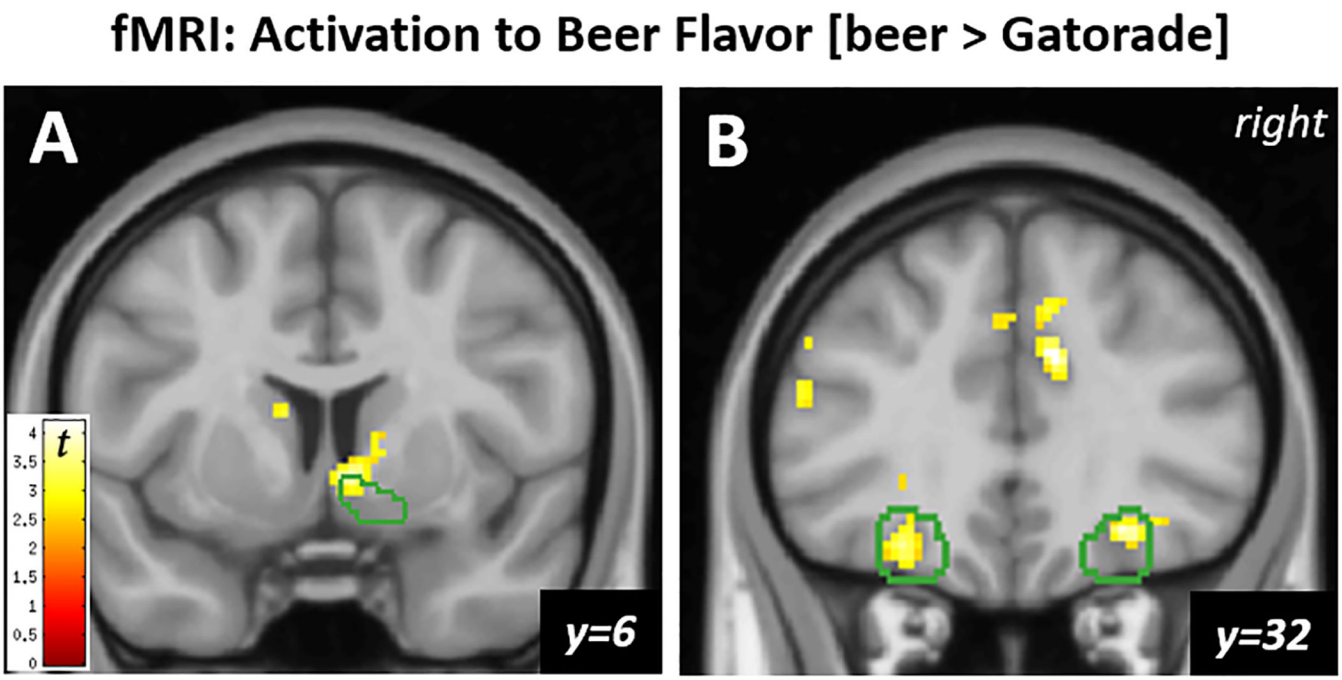

Figure 3. Response to beer flavor compared to appetitive control

Voxelwise *t*-statistic map illustrating (A) right VST and (B) orbitofrontal cortex (OFC) BOLD response to alcohol flavor CS in n=28 male drinkers. Search regions are outlined in green. Effects illustrated at a voxel-wise display threshold, p<0.01, uncorrected; k=100.

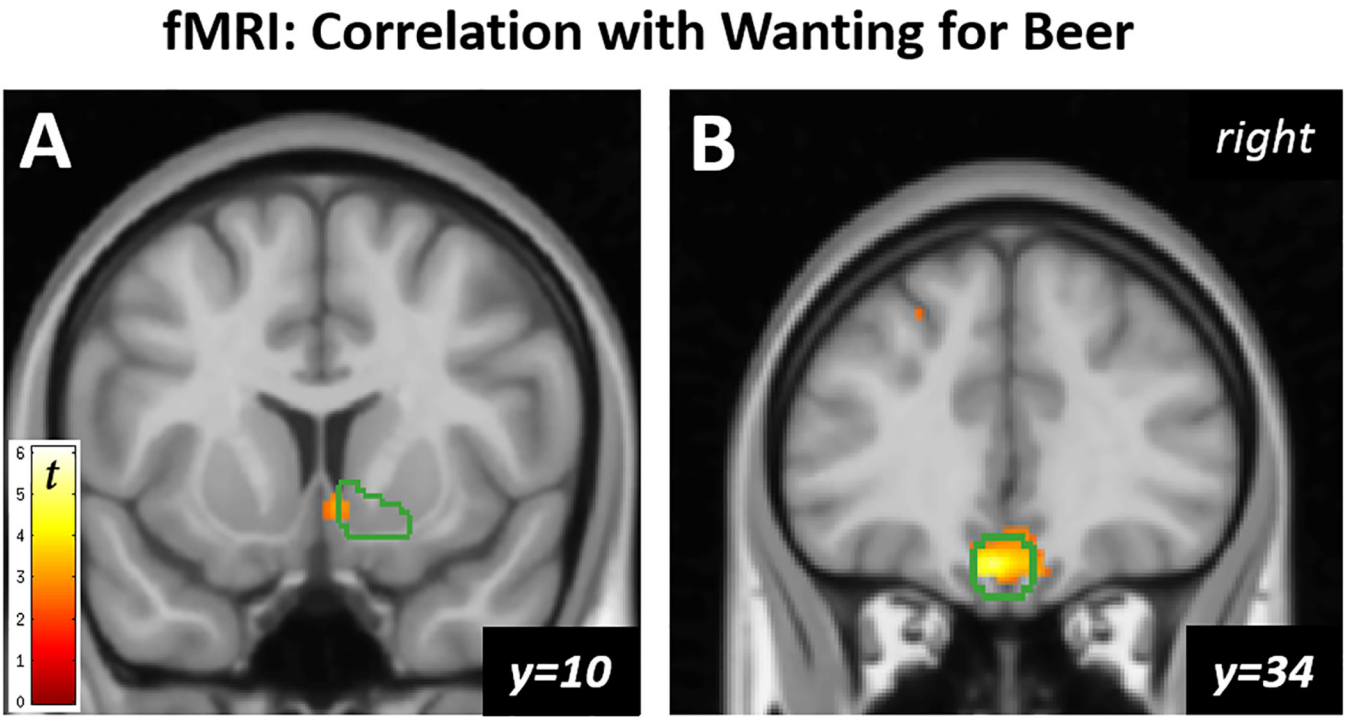

Figure 4. Correlations between wanting and [beer flavor > Gatorade] BOLD contrast

Voxelwise *t*-statistic map shows significant positive correlation with "number of beers wanted" in (A) right VST and (B) medial OFC (search regions in green). Effect illustrated at a display threshold, $p<0.01$, uncorrected; $k=100$.

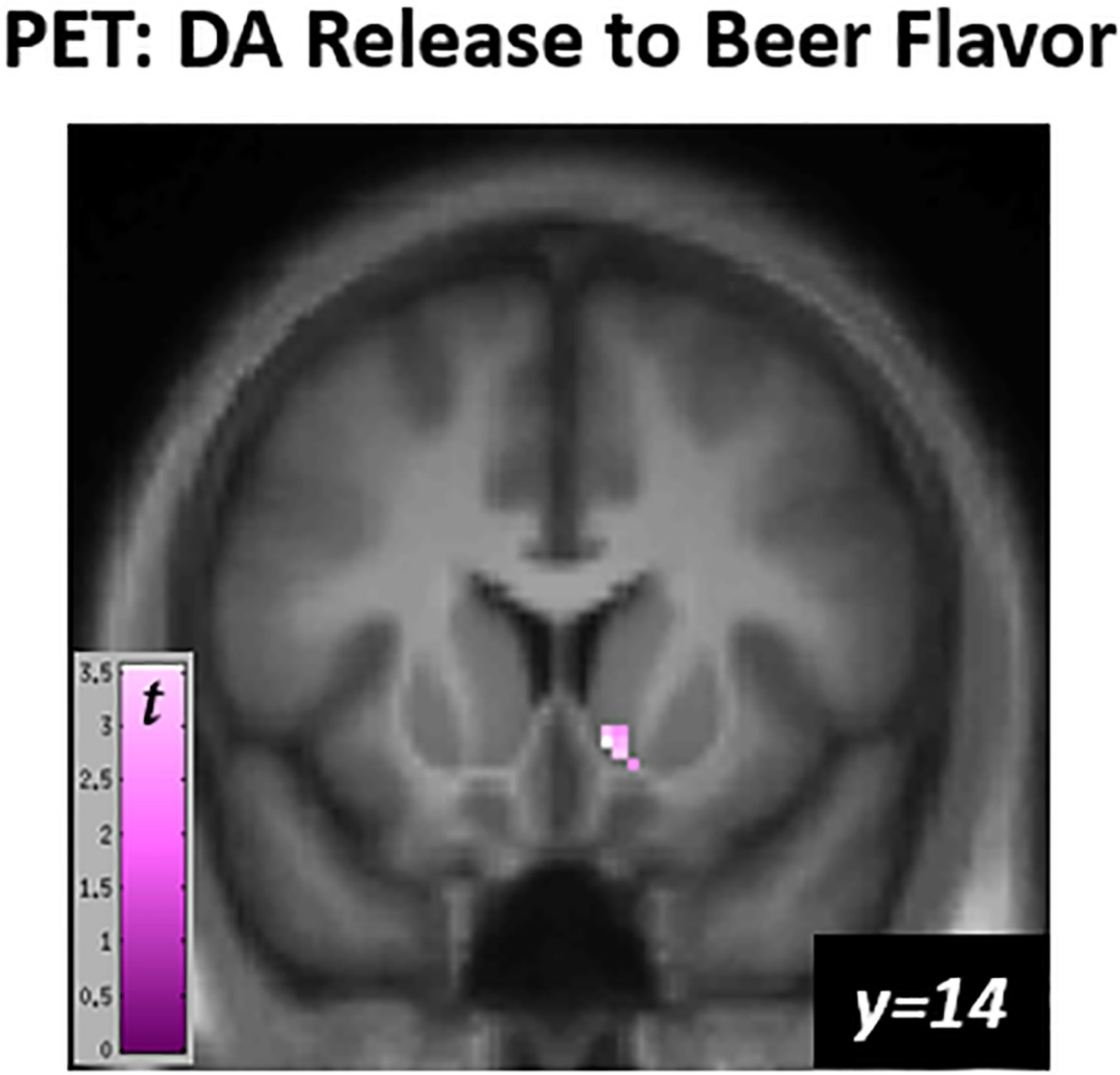

Figure 5. PET

Right VST DA response to beer flavor compared to Gatorade [$\Delta BP_{ND} > 0$] in (n=28) male drinkers, display height threshold p<0.01, uncorrected, k=5.

Table 1

Subject Characteristics (n = 28)

	Mean (SD)	Range	*n*(%)
Age	23.8 (2.3)	21–29	
Caucasian	-	-	28(100%)
Education	16.0 (1.3)	12–19	
Drinks per week[1,a]	15.8 (10.4)	2–37	
Drinks per drinking day[1,a]	5.0 (3.0)	1–10	
Heavy drinking days per week[1,2,a]	1.5 (1.4)	0–5.7	
AUDIT[3]	10.7 (6.2)	3–28	

[1]From the Timeline Followback (TLFB) Interview.

[2]Five or more drinks per day.

[3]Alcohol Use Disorders Identification Test.

[a]From the interview, preceeding PET. TLFB Interview data on fMRI study day: Drinks/week 14.6 (9.6); Drinks/drinking day 4.8 (2.9); Heavy drinking days/week 1.3 (1.3)

Table 2

BOLD responses in appetitive flavor contrasts[1]

			MNI coordinate (mm)		
Region	**Cluster size *k***	**Peak *Z***	***x***	***y***	***z***
[beer > Gatorade]					
R anterior cingulate cortex	30	4.14	**10**	**32**	**30**
		3.30	12	24	30
L fusiform cortex	13	4.00	**−38**	**−48**	**−8**
R ventral striatum	15	3.40*	**6**	**2**	**−6**
R OFC	10	3.39*	**22**	**36**	**−14**
R anterior insula/FO	14	3.29	**34**	**24**	**−16**
		3.16	36	28	−8
[Gatorade > beer]					
R postcentral gyrus	16	3.83	**54**	**−12**	**34**
R postcentral gyrus	10	3.57	**60**	**−16**	**48**

[1]Compared to water baseline. Voxel-wise height threshold, $p_{uncorr} < 0.001$, $k = 10$.

*$p_{FWE} < 0.05$, corrected for *a priori* region of interest.

MNI, Montreal Neurological Institute; R, right; L, left; OFC, orbitofrontal cortex; FO, frontal operculum.

Table 3

Correlation coefficients by modality in right VST[1]

	BP_{beer}	$BP_{Gatorade}$	ΔBP
[beer > water]	0.05	0.05	−0.03
[Gatorade > water]	−0.16	−0.31	−0.21
[beer > Gatorade]	0.18	0.30	0.15

[1] Spatial extent defined by cluster exceeding $p_{uncorr}<0.01$ within the anatomical VST.

Pearson's *r* for the correlation between binding potential (BP) of [^{11}C]raclopride (columns, PET) and flavor contrasts (rows, fMRI). All *p*s > 0.1.